CHAPTER 1.5 - WET Data Review and Interpretation

This chapter describes the WET test review process generally followed by the Biomonitoring Coordinator. This chapter is provided for staff, permittees, and others who want to understand why review decisions were made; it also discusses common data patterns and provides guidance on dose-response, confidence intervals, and other information.

NOTICE: This document is intended solely as guidance, and does not contain any mandatory requirements except where requirements found in statute or administrative rule are referenced. This guidance does not establish or affect legal rights or obligations, and is not finally determinative of any of the issues addressed. This guidance does not create any rights enforceable by any party in litigation with the State of Wisconsin or the Department of Natural Resources. Any regulatory decisions made by the Department of Natural Resources in any matter addressed by this guidance will be made by applying the governing statutes and administrative rules to the relevant facts.

"Whole Effluent Toxicity (WET) Test Report Forms"

According to the "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd edition*" (Methods Manual), WET Test Report Forms are required to be submitted for demonstrating test completion and compliance with a WPDES permit. The WET Test Report Form and instructions for completing the form can be found in Section 6 of the Methods Manual. The original, complete, signed version of the WET Test Report Form must be sent to the Biomonitoring Coordinator (Bureau of Watershed Management, Department of Natural Resources, 101 South Webster St., P.O. Box 7921, Madison, WI 53707-7921) by the date specified in the WPDES permit. Report forms and attachments (if applicable) must contain all information needed to determine compliance with the requirements specified in the Methods Manual. Reports must contain a description and justification of any abnormal procedures, conditions, or manipulations used in the test(s). The permittee and/or laboratory should also provide any attachments or additional information which they believe to be relevant to the test. All other test documentation (e.g. bench sheets, record books, etc.) needed to fulfill manual or QA requirements must be maintained at the laboratory for laboratory certification purposes (see Section 3.16, of the Methods Manual).

WET Test Report Forms should include observations made by the permittee or lab that may influence test results or data interpretation, such as: 1) unusual conditions (e.g., plant upsets, slug loads, weather conditions, etc.) during sampling periods, 2) deviations from test specifications or any sample manipulation (aeration, filtration, addition of chemicals, etc.) that is determined to be necessary for successful completion of a test, and/or 3) unusual behavior or appearance of test organisms (e.g., young developed in the brood pouch of the adults, but not released during the exposure period; partially or fully developed young released, but all dead at the end of the 24-h period; lethargy, hyperactivity, spots or filaments, discoloration, excessive ventilation, etc.).

Department Routing Procedures for the WET Test Report Form

The Methods Manual requires that WET Test Report Forms be sent directly to the Biomonitoring Coordinator. Upon receipt at the central office, forms are date-stamped so others can determine when they were received. Report forms are then reviewed as soon as possible. The Biomonitoring Coordinator sends copies of the reviewed form to Basin Engineers (and sometimes Permit Coordinators) for tracking with permit conditions. If qualified staff are not available in the central office to review WET reports, administrative staff should be informed so that reports can be date stamped and immediately sent to Regional staff upon receipt.

Quality Assurance And Data Review And Reporting Process

A thorough WET data review process includes many steps, including those described below.

Review and confirmation of test conditions - The Methods Manual requires a list of conditions (e.g., test

temperature, number of replicates, test chamber size and volume, lighting, feeding regimes, etc.), for each acute and chronic test, that must be followed in all tests submitted for WPDES compliance. Before submitting results to the Department, the results of each test should be reviewed by permittees and lab staff to ensure that conditions were met within the flexibility provided by the Methods Manual. Labs should verify daily measurements to ensure that values are within the acceptable ranges allowed by the test methods and report whether these conditions were met on page 1 of the WET Test Report Form. Any deviations from Methods Manual requirements should be clearly reported on the report form. The Biomonitoring Coordinator will check and attempt to verify this information while reviewing WET test data.

Review of reference toxicant testing – Reference toxicant tests, conducted according to Section 3.15 of the Methods Manual, are conducted under the same conditions (e.g., test duration, test conditions, test endpoint) as effluent tests, substituting a known toxicant for effluent samples. Reference toxicant testing is an important quality control practice that is required in order to 1) determine the sensitivity of the test organisms over time, and 2) assess the quality and comparability of within- and between-laboratory test results. Reference toxicant test results can be used to help identify potential sources of variability, such as test organism health, difference among batches of organisms, changes in laboratory water or food quality, and performance by laboratory technicians. By standardizing reference toxicants, testing laboratories can compare test results within their own lab and permittees and the Department can compare and evaluate laboratories. (See Chapter 2.1 for guidance on how to evaluate reference toxicant data when choosing a new WET lab.) Labs must indicate on page 1 of the report form whether reference toxicant tests that were performed at the time of effluent testing were within acceptable limits.

Reference toxicant testing is required on at least a monthly basis for each test method routinely conducted in a laboratory. A "control chart" is required to be prepared for each combination of test species and test condition. A control chart is a running plot that is maintained for the toxicity values from successive tests with the reference toxicant, and endpoints (LC_{50} or IC_{25}) are examined to determine if they are within prescribed limits. Control charts are used to evaluate the cumulative trend of results from a series of samples. The mean and upper and lower control limits (± 2 S.D.) are re-calculated with each successive test result. After two years of data collection, or a minimum of 20 data points, the control chart is maintained using only the 20 most recent data. Reference toxicant test data are reviewed to look for outliers, which are values falling outside the upper and lower control limits, and trends of increasing or decreasing sensitivity.

WET test review usually includes an evaluation of the most recent reference toxicant test and the control charts maintained by the laboratory. The Methods Manual requires that certified labs complete monthly reference toxicant tests and submit results on a quarterly basis, in most cases. If the Department determines that a series of reference toxicant tests are out of the acceptable range, effluent testing conducted during the same period may be rejected. Lab performance is expected to improve with experience and control limits generated by experienced, quality labs may gradually narrow over time. Highly proficient laboratories which develop very narrow control limits may be unfairly penalized if a test which falls just outside the control limits is rejected *de facto*. For this reason, the width of control limits should be considered in determining whether or not data is to be rejected.

Review of Water Chemistry Data - Receiving water and effluent data for hardness, alkalinity, pH, total ammonia, and total residual chlorine are entered on page 1 of the WET Test Report Form, so that general sample characteristics can be assessed to determine their potential impact on test results. The values reported for hardness, alkalinity, and ammonia should be from sample measurements taken upon arrival at the lab. The values reported for Total Residual Chlorine and pH should be from measurements taken after samples have been warmed to test temperatures and just prior to use in tests.

Sometimes hardness, alkalinity, and ammonia data can be extremely important for determining whether test or effluent conditions have impacted test results. Test organisms may experience reduced survival and/or reproduction

if exposed to test waters that have hardness or alkalinity values significantly different from culture waters. Total ammonia and pH values should be reviewed to determine whether ammonia could have caused toxicity. (In general, ammonia levels > 10 mg/l in an effluent sample are a concern, if pH is above 8.0; at lower pHs, more ammonia is needed to cause toxicity.)

Review of test acceptability criteria - The Methods Manual provides method-specific minimum criteria that must be met in order for WET tests to be acceptable (e.g., minimum survival, reproduction, growth, variability in controls, etc.). These criteria are set in order to insure that any effects noted during WET tests are due to the effluent being tested, and not due to dilution water, lab error, or other factors. The WET Test Report Form provides spaces where it must be reported whether these criteria were met during each test. Tests not meeting the minimum test acceptability criteria may be considered invalid and need to be repeated (see Section 3.8 and 3.9 of the Methods Manual for details).

Review of test variability - The variability of each WET test, measured as percent coefficient of variation (%CV) between replicates, is required to be calculated and reported with all test results. It is also recommended that laboratories maintain control charts for test variability. These control charts allow laboratories to assess individual test variability in the context of typical variability within the laboratory. High test variability can result in insensitive tests or unexpected dose-response relationships. Consult Chapter 2.9 & USEPA (2000) for additional guidance on WET test method variability.

Review of test results and dose-response relationships – Acute and chronic WET test results must be presented tabularly and graphically on WET Test Report Forms (see pages 2 and 3 of the form). Replicate and mean survival, growth, and reproduction data for the control and effluent treatments are given in tables and are plotted against the concentrations tested in graphs found at the bottom of each test results section on the form. Graphs give a visual picture of the dose-response, variability of the data, and suspicious data or potential outliers.

The concept of a dose-response relationship is a fundamental concept in toxicology. This concept assumes that there is a relationship between the dose (or concentration) of a toxicant and a measured response. The dose-response concept is the basis for the determination of point estimates (LC_{50}, IC_{25}) in WET testing. A biological response (mortality, growth or reproductive inhibition) is measured at a range of effluent concentrations to develop a dose-response curve. In general, more severe responses are expected to occur at higher concentrations of toxicant, and less severe responses at lower concentrations. (See Figure 1.5.1 below.)

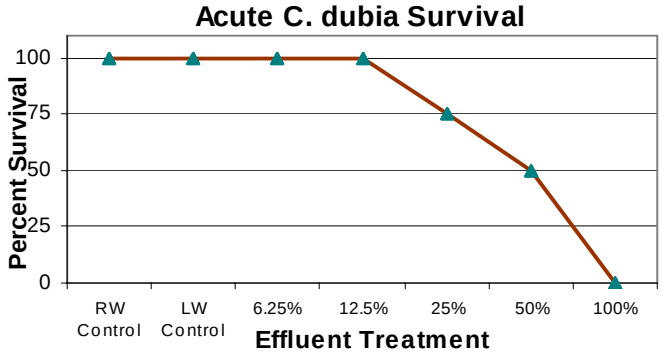


Figure 1.5.1. Example "normal" dose-response when toxicity is present.

Test endpoints (acute: LC_{50}; chronic: IC_{25}), determined using computerized statistical packages (see Section 5 of the Methods Manual), are also reported and compared to compliance criteria to determine test pass/fail. In most cases, an acute test is considered a "pass" if the $LC_{50} \geq 100\%$ effluent. A chronic test is considered a "pass" if the IC_{25} > the

instream waste concentration (IWC), which is a site-specific estimate of the proportion of effluent to receiving water. (See Chapter 2.4 for a discussion regarding LC_{50}, IC_{25}, and TU values; Chapter 1.3 discusses the IWC.)

How Is The Dose-Response Concept Used In WET Testing?

Guidance is given here which may be used when evaluating the dose-response relationship as a part of the data review and reporting process. Provided are examples of common patterns in WET test data, possible causes and solutions for unexpected patterns, and guidance on when data may be accepted or rejected based on the dose-response (or lack thereof). It should be noted that the determination of a valid dose-response relationship is not always clear cut. Data from some tests may suggest consultation with a professional toxicologist (such as the Department's Biomonitoring Coordinator or staff at the State Lab of Hygiene and other certified WET labs). Tests that exhibit unexpected dose-response relationships may indicate a need for further investigation and possible retesting. In general, when unexpected or apparently anomalous dose-response relationships are encountered, the following is recommended:

- **Attempt to determine a cause for the response** - Test review and specific guidance (such as "*What are some patterns of dose-response relationships typically seen in WET test data?*", below) may assist in determining a cause for an unexpected dose-response. Unexpected responses could be valid patterns or anomalies resulting from Type I test error (e.g., lab error, sampling problems, etc.), high test variability, or other causes. If a given effluent consistently produces an "unexpected" dose-response, there is likely a physical, chemical or biological cause. In situations where difficult-to-interpret dose-response relationships are produced consistently by a given effluent, consultation with professional toxicologists is recommended.

- **Follow guidance for specific dose-response patterns** - Detailed guidance is given below which describes dose-response curves and provides examples of 10 patterns that may be exhibited by WET test data. This section provides guidance in interpreting each dose-response pattern using a step-by-step review process. Based on this review, the guidance may recommend acceptance of the calculated results as valid and reliable, explanation of the calculated results as anomalous, or retesting.

- **Coordination between the DNR, permittee, and laboratory** - It is often wise for the DNR, permittee, and laboratory personnel to work together to resolve difficult-to-interpret WET test data. Discussions should be initiated as soon as possible when questions arise regarding WET test results.

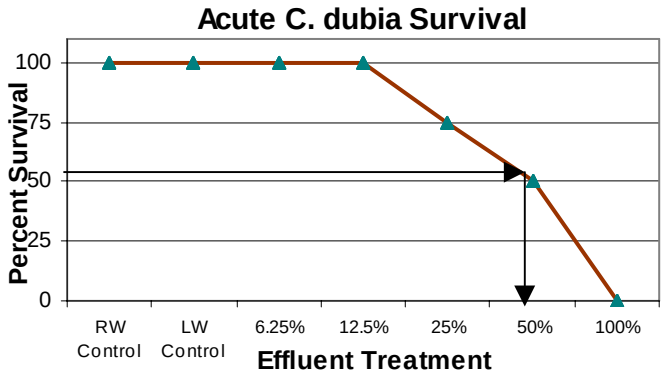


Figure 1.5.2. From the dose-response curve, a "point estimate effect concentration" (LC_{50}) can be calculated. In this example, LC_{50} = 50% effluent.

The dose-response concept is the basis for the determination of point estimates in WET testing. A biological response (mortality, growth inhibition, etc.) is measured at a range of effluent concentrations to develop a dose-response curve. From the resulting dose-response curve, a "point estimate effect concentration" (e.g., LC_{50}, IC_{25})

can be calculated (see Figure 1.5.2). The effect concentration is an estimate of the concentration of effluent that will produce a specific level of response (e.g., 50% mortality, 25% inhibition).

One assumption of this concept is that every toxicant should exhibit a dose-response relationship, assuming the appropriate response is measured and the concentration range evaluated is appropriate. Use of this concept can be helpful in determining whether an effluent possesses toxicity and in identifying anomalous test results. Tests that exhibit unexpected dose-response relationships may indicate a need for further testing. As noted above, if a given effluent consistently produces an "unexpected" dose-response, there is likely a physical, chemical or biological cause which should be further investigated.

Common Dose-Response Relationships

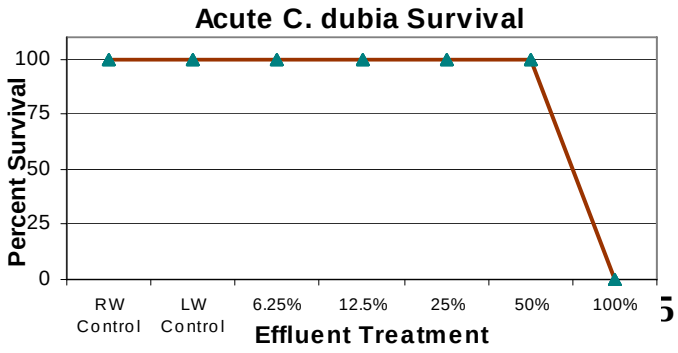
Ten dose-response patterns that may appear in WET testing are described and illustrated below using hypothetical test data. This section provides guidance in interpreting each dose-response pattern. The guidance focuses on determining a cause for unexpected dose-response patterns by recommending a step-by-step review process. Based on this review, the guidance may recommend acceptance of the calculated endpoints (LC_{50} or IC_{25}) as valid and reliable, explanation of the calculated results as anomalous, or retesting. When retesting is recommended, this generally means beginning a new test on a newly collected sample since sample holding times are expired by the time results are obtained from the original test. Test results should be reported for all tests conducted, even if retesting is recommended.

This guidance on dose-response relationships is for informational purposes only, and it is not the intent of this chapter to recommend the frequent disqualification or repetition of WET tests. Several warnings and safeguards should be considered when implementing the guidance in this chapter. First, unexpected dose-response relationships should not occur with any regular frequency. Second, it is not recommended to reject only those tests in which toxicity is found at or below the concentration of concern. If screening is to be done for unexpected dose-response relationships, all tests should be screened in a similar manner. Third, all results should be reported to and reviewed by the Department (including those disqualified and repeated). In most cases, the DNR should make final decisions about whether test results are to be rejected based on unusual dose-response patterns.

1) **Ideal dose-response relationship.** This response pattern (see Figure 1.5.1) shows a clear dose-response relationship, with multiple effluent concentrations identified as significantly different from the control. This pattern shows a monotonic decrease in response, meaning that the response steadily decreases for each higher effluent concentration. This pattern is indicative of a well designed test with appropriately chosen concentrations that bracket the effluent's range of toxicity. Under these circumstances, point estimation techniques (LC_{50}, IC_{25}) recommended in the Methods Manual should provide reliable results.

2) **All or nothing response.** The "all or nothing" response pattern is very common in WET test data. This response pattern (Figure 1.5.3) is characterized by a transition from no significant effect at one effluent concentration to a complete effect (e.g., 100% mortality) at the next higher concentration. This pattern also represents a valid dose-response relationship and point estimation techniques recommended in the Methods Manual should provide reliable results. This pattern of response is indicative of a steep dose-response curve for the given effluent, and under these circumstances, the precision of the estimate may be improved by closer spacing of effluent concentrations or the addition of intermediate concentrations in future testing.

Figure 1.5.3 An example dose-response, showing an "all or nothing" response.



3) **Stimulatory response at low concentrations and detrimental effects at higher concentrations.** A stimulatory response is a non-monotonic dose-response relationship characterized by a measured increase in the response at low concentrations. This stimulation at low concentrations can be followed by a detrimental effect at higher concentrations (see Figure 1.5.4) or by no effect at higher concentrations (see #4 below). The stimulatory pattern characterized in Figure 1.5.4 is typically found with chronic, sublethal endpoints such as reproduction and growth. For instance, test organism reproduction may increase (relative to the control) at low concentrations of an effluent and decrease relative to the control at higher concentrations. This dose-response pattern, while non-monotonic, is still a valid dose-response relationship, and point estimation techniques required by the Methods Manual should provide reliable results.

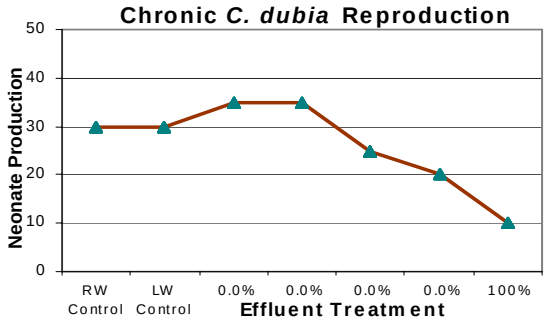


Figure 1.5.4 An example dose-response, showing a stimulatory response at low concentrations and detrimental effects at higher concentrations

4) **Stimulation at low concentrations but no significant effect at higher concentrations.** This dose-response relationship is similar to the previous example in that stimulation is observed at lower concentrations, but in this case, higher concentrations do not produce significant effects (see Figure 1.5.5). Results from point estimation techniques should be interpreted carefully when this response pattern is encountered, because the inhibition concentration percentage (ICp) procedure may produce effect concentrations that indicate toxicity at effluent concentrations where the response is comparable to the control response.

The ICp procedure assumes that responses: 1) are from a random, independent, and representative sample of test data; 2) follow a piecewise linear response function; and 3) are monotonically non-increasing, meaning that the mean response for each higher concentration is less than or equal to the mean response for the previous concentration. If the data are not monotonically non-increasing, the ICp procedure adjusts the response means using a "smoothing" technique that averages adjacent means. This technique averages response means (including that of the control) with those of the next highest test concentration until responses are monotonically non-increasing. In cases where the responses at the low effluent concentrations are much higher than in the control, the smoothing process may result in a large upward adjustment in the control mean. This can lead to an IC_{25} result that is less than the highest test concentration, even though the highest test concentration was not statistically different from the control treatment and even if a percent difference of less than 25% was observed between the control response and the response at the highest test concentration. If the response pattern shown in Figure 1.5.5 is encountered, the following steps should be taken in addition to standard test review procedures:

a) **Evaluate the concentration range** - If the highest concentration used in the test was less than 100% effluent, future tests on this effluent should include higher test concentrations to establish if a valid dose-response relationship exists. This may not be necessary if the IWC is much lower than 100% effluent and test results indicate no toxicity at that level and above.

b) **Evaluate control response** - It is possible that the response pattern shown in Figure 1.5.5 could result from poor control performance rather than stimulation at lower effluent concentrations. Poor control performance could cause a toxic effect at higher effluent concentrations not to be detected. To evaluate this possibility, compare the control response to the normal control performance for the laboratory. If (1) the test exhibits a response pattern similar to that shown in Figure 1.5.5 and (2) the control response is well below the laboratory's normal range of control performance, then retesting is recommended even if the minimum test acceptability criteria have been met. For example, if a laboratory usually achieves a control mean of 25-30 neonates in the *C. dubia* chronic test, a control mean of 15-18 neonates (in conjunction with a non-ideal dose-response curve) would warrant retesting. In this situation, suppressed control performance could be considered as the cause for this response pattern rather than stimulation. A review of control performance should also investigate the possibility of poor performance in a single replicate substantially reducing the mean control response. In this case, retesting is also recommended.

c) **Evaluate the ICp calculation** - If a test exhibits the pattern shown in Figure 1.5.5 and it has been determined that the pattern is not due to poor control performance, then discrepancies may be due to bias from the ICp smoothing technique. To determine if this is the case, calculate the percent difference between the response at the IWC and the control ([*mean response at IWC/mean control response] x 100*). If the observed percent difference between the response at the IWC and the control is < 25% and the response at the IWC is not statistically significantly different from the control response, then a calculated IC_{25} of less than the IWC should be noted as anomalous and the effluent determined to be non-toxic at the IWC. If the observed difference is $\geq$ 25%, then the calculated IC_{25} should be considered valid.

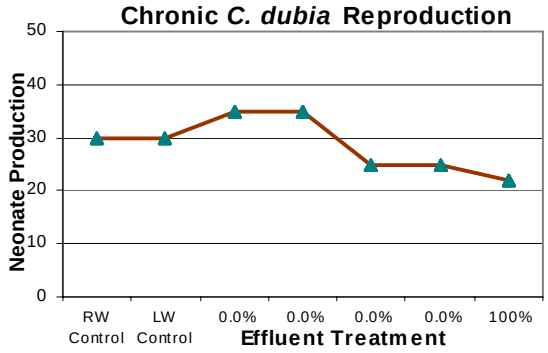


Figure 1.5.5 An example dose-response, showing a stimulatory response at low concentrations but no significant effect at higher concentrations

5) **Interrupted dose-response: significant effect bracketed by non-significant effects.** This response pattern is characterized by a single test concentration showing a significant difference from the control while adjacent higher and lower test concentrations do not differ significantly from the control (Figure 1.5.6). When this response pattern is encountered, point estimation techniques generally will yield reliable results.

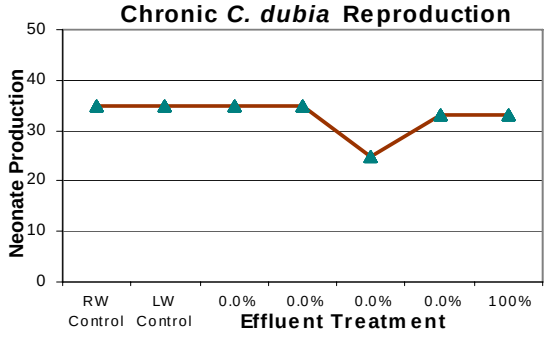


Figure 1.5.6 An example dose-response, showing an Interrupted dose-response: non-significant effects bracketed by significant effects

6) **Interrupted dose-response: non-significant effects bracketed by significant effects.** This response pattern is similar to the previous pattern in that the dose-response curve is interrupted, however, this pattern is characterized by two or more concentrations showing a significant difference from the control while an intermediate test concentration does not differ significantly from the control (Figure 1.5.7). When this response pattern is encountered, point estimation techniques will generally yield reliable results.

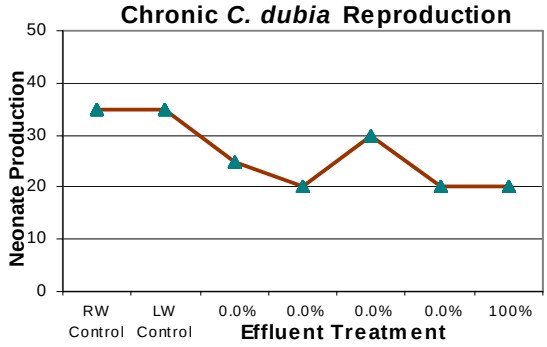


Figure 1.5.7 An example dose-response, showing an Interrupted dose-response: non-significant effects bracketed by significant effects

7) **Significant effects only at highest concentration.** This response pattern is characterized by only the highest test concentration producing a significantly different response from the control (see Figure 1.5.8). This response pattern should be considered to be a valid dose-response relationship and results determined by point estimation should be assumed to be reliable.

When the response pattern shown in Figure 1.5.8 (significant effects only at highest concentration) occurs, the concentrations used for testing should be evaluated in future tests using this effluent (especially in chronic tests where the highest concentration is at or near the IWC). This would provide a better opportunity to confirm a dose-response relationship that may exist at the upper end of the concentration range. This approach should be used only if historical testing of the effluent indicates consistency and the effect concentration (e.g., IC_{25})is not likely to fall below the adjusted test concentration series.

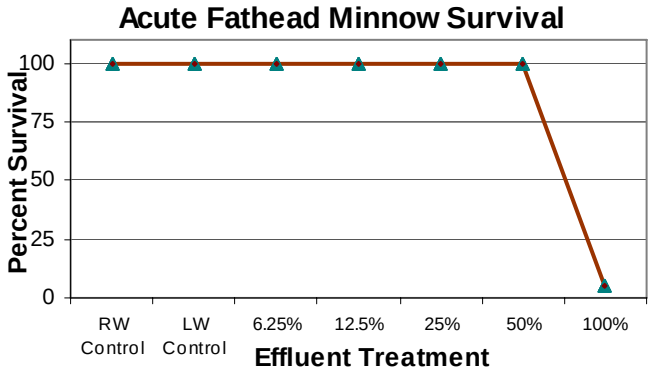


Figure 1.5.8 An example dose-response, showing significant effects only at highest concentration

8) **Significant effects at all test concentrations but flat dose-response curve.** This response pattern is

demonstrated in Figure 1.5.9. All of the test concentrations produce a response that is significantly different from the control response, but a clear dose-response relationship cannot be determined. This response pattern could be due to: (1) extremely low variability in the control, (2) an unusually high control response, (3) an inappropriate dilution water and improper use of dilution water controls, (4) inappropriate test dilution series, (5) potential pathogen effects in the effluent, (6) an unusual effluent-dilution water interaction.

The following actions should be taken to determine a cause for this dose-response pattern and to subsequently determine the validity of calculated results:

a) **Evaluate control response** - The dose-response pattern shown in Figure 1.5.9 could result from an unusually high response in the control treatment. Laboratories are encouraged to track the performance of controls in tests conducted over time. When the response pattern shown in Figure 1.5.9 is exhibited, the control response for the test should be compared to historic control performance in the laboratory using the given dilution water. If the mean control response is above the normal range for that laboratory and dilution water, it may be wise to repeat the test.

b) **Evaluate dilution water** - The improper use of dilution waters and controls could cause the dose-response pattern shown in Figure 1.5.9. It should be confirmed that test concentrations were compared to the dilution water control and not a culture water control.

c) **Consider pathogen effect** - The dose-response pattern shown in Figure 1.5.9 could also be due to the presence of pathogens in the effluent. The most common identifier of pathogen effects are sporadic mortalities and extremely high variability between replicates. The pathogen effect is more common in tests using fish species than in invertebrate testing and in chronic tests than acute tests. If within-treatment CVs for survival are >40% for effluent concentrations and relatively small for control replicates in standard synthetic water, a pathogen effect should be considered. If a pathogen is suspected in the effluent, this may be confirmed in subsequent side-by-side testing using the effluent and the effluent treated by brief exposure to UV light or the addition of antibiotics, or increasing the number of replicates and using less test organisms in each replicate. If pathogen effects in the effluent are confirmed, the sample should be retested.

d) **Continued testing** - If all of the above scenarios have been investigated and have not revealed the cause of the response pattern, the results should be considered valid; however, continued testing should be initiated in an effort to identify the cause of the response pattern. If an effluent consistently exhibits this response pattern, additional investigations could include chemical analysis or initiation of toxicity identification procedures.

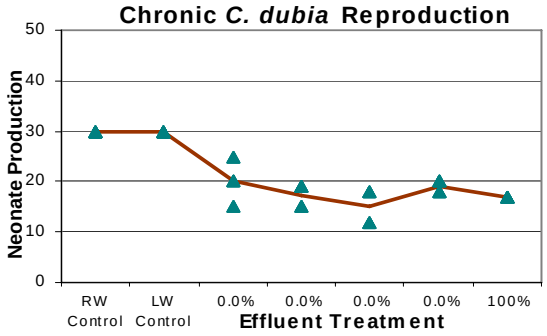


Figure 1.5.9 An example dose-response, showing significant effects at all test concentrations but flat dose-response curve

9) **Significant effects at all test concentrations with a sloped dose-response curve.** This dose-response pattern is similar to the pattern identified in item #8 above except a dose-response curve can be identified at the higher effluent concentrations (Figure 1.5.10). This pattern is considered to be a valid dose-response relationship, and point estimation techniques will generally yield reliable results.

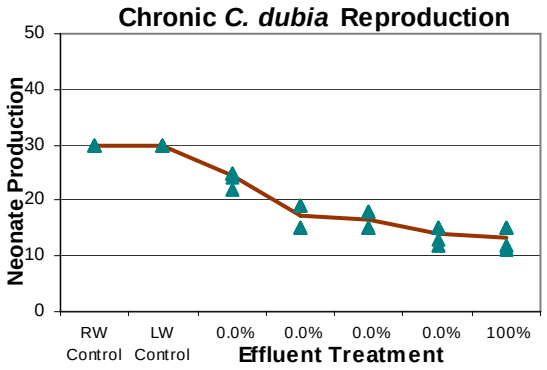


Figure 1.5.10 An example dose-response, showing significant effects at all test concentrations with a sloped dose-response curve

10) **Inverse dose-response relationship.** This response pattern is characterized by a relationship in which adverse effects decrease with increasing effluent concentration (Figure 1.5.11). This situation is most often encountered in algal growth tests and sometimes in *C. dubia* chronic tests, and is typically caused by excess nutrients in the effluent. An inverse response pattern could also be due to the presence of pathogens in the effluent. While a valid dose-response relationship is demonstrated in this circumstance, the effluent may be nontoxic since the direction of the dose-response relationship indicates decreasing adverse effects. It should be noted that while the effluent may be non-toxic, the presence of excess nutrients still may pose a potential risk to the environment due to nutrient enrichment and oxygen depletion.

An inverse dose-response pattern also may occur in tests when the dilution water used is a receiving water or synthetic water adjusted to approximate the receiving water characteristics. In such situations, the inverse dose-response pattern can result from toxicity in the receiving water or the limitation of necessary components (i.e., hardness) in the receiving water or adjusted synthetic water. Under such circumstances, the objective of the toxicity test should be evaluated. If the objective of the test is to determine the toxicity of the effluent in the natural receiving water, then the results indicate no toxicity in the sample. If the objective of the toxicity test is to determine the absolute presence of toxicity in the effluent, the sample should be retested using a standard synthetic dilution water.

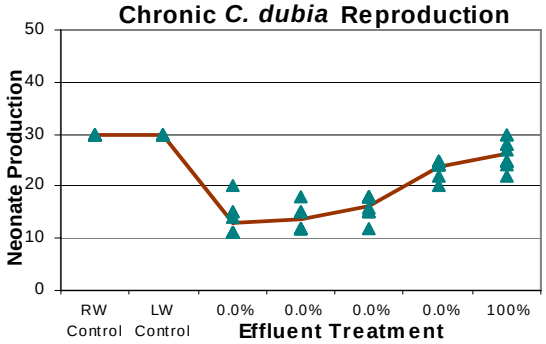


Figure 1.5.11 An example dose-response,

Confidence Intervals

The Methods Manual requires that test endpoints be reported as an LC_{50} (acute) or IC_{25} (chronic). The 95% confidence intervals associated with these endpoints must also be reported, as an estimate of the precision (uncertainty) around the LC_{50} or IC_{25} value. The Methods Manual requires that the proper statistical method be performed using EPA or commercially available software, which generally produce a point estimate with the associated 95% confidence intervals. It is important to note that under certain circumstances confidence intervals are not produced by the software or are unreliable (e.g., if test data do not meet specific assumptions required by the statistical methods, if point estimates are outside of the test concentration range, or if specific limitations of statistical software are encountered). Confidence intervals are not used when determining compliance, but must be reported (when available) and may be used as supplemental information when interpreting test results.

The 95% confidence intervals are a measure of the uncertainty of the endpoint calculated by the statistical package. As the 95% confidence intervals of the point estimate increases (i.e., get wider), the uncertainty in that estimate of the statistical endpoint increases. Conversely, the smaller the width of the confidence intervals, the more certain one can be that the endpoint determined by the statistical program is accurate.

In WET testing, confidence intervals can be a measure of intratest variability. The confidence intervals for chronic endpoints are directly influenced by the variability between replicates in each treatment and the model used to interpolate the point estimate. The confidence intervals for acute test results using a point estimate approach, however, are not influenced by variability between replicates but by the characteristics of the dose-response relationship. As discussed in Chapter 2.12 of this guidance document, the certainty in point estimates is also a function of the dilutions tested and their proximity to the actual statistical endpoint being calculated. One will get a better estimate of the LC_{50} (tighter confidence intervals) if dilutions are tested near the concentration which actually results in 50% mortality.

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